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ON THE PERFECT STAGE OF SCLEROTIUM ROLFSII SACC. PRODUCED ON CULTURE MEDIA

PRELIMINARY REPORT

By

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ON THE PERFECT STAGE OF SCLEROTIUM ROLFSII SACC. PRODUCED ON CULTURE MEDIA

PRELIMINARY REPORT *

Kazuo Goto

(Accepted for publication August 27, 1930)

A sclerotial disease due to *Sclerotium Rolfsii* Sacc. is worldwide in distribution and has a wide range in host relations including a large number of cultivated plants on which an injurious nature is often prevalent. Consequently an extensive literature has been accumulated about the pathological as well as physiological studies on this subject, nevertheless references to its sporulation is rather limited. In 1919 Sawada[28] for the first time recognized *Hypochnus centrifugus* (Lév.) Tul. as a perfect stage of *Sclerotium Rolfsii*, though he found this relation only in nature, and further included *Hypochnus Cucumeris* Fr. and *Hypochnus Solani* Prill et Del. under this binomial. Nakata[19] studying a large number of the fungus in question, found a certain fruiting structure developed on some infected sugar beets and "Konjac" plant (*Amorphophallus Konjac*) which had been inoculated with three different strains, as well as on some cultures of the mutants which had been derived from his cultures of *Sclerotium Rolfsii*[20] and came to a conclusion that *Sclerotium Rolfsii* might be an imperfect stage of *Hypochnus centrifugus* as proposed by Sawada, but the latter could not be considered as being identical with *Hypochnus Solani* or *Hypochnus Cucumeris*.

It is curious, however, that as to the relationship between these two stages very few authentic arguments have been offered by phytopathologists other than the above-named compatriots. According to Higgins[13] the fungus must have relationship to Basidiomycetes, as the hyphae have clamp connections and each hyphal cell contains two nuclei. Taubenhaus[33] found some minute bodies developed on an affected fruit of watermelon, and considered them as abortive fruiting structures, but no mention was given as to the taxonomy of the fungus. According to Nakata[20] Whetzel stated in a letter to Nakata that he was wondering whether the fruiting structure observed by Nakata was really a perfect stage of the fungus in question, or due to the contamination by *Corticium vagum*.

It is the purpose in this paper to describe more fully the fruiting structure of the fungus which was secured by the writer in the course of his

* Contributions from the Phytopathological Laboratory, Taihoku Imperial University Formosa, Japan, made under the direction of Prof. T. Matsumoto.

cultural studies on this organism, and at the same time to discuss briefly about the taxonomy of the fungus in question.

The writer wishes to acknowledge his indebtedness to Prof. T. MATSUMOTO for his careful direction during the course of the study as well as in the preparation of this paper, and also to Assist. Prof. Yoshimatsu YAMAMOTO for his kind help in identifying some host plants.

MATERIALS

The strains used in the present investigation were secured by removing sclerotia from affected areas and transferring them to sterile culture media in the manner usually employed in ordinary cultural works.* In the case of R 24, however, a small piece of hymenia was used. Up to the present time the sporulation took place more frequently on culture media prepared by decocting 300 grams of fresh onion in 1000 cc. distilled water, followed by adding 23 grams agar. More details about the strains will be shown in the following protocol.

No. of cultures	Isolated from	Locality	Date isolated
R 1.	*Allium fistulosum* L.	College exp. farm.	July 29. 1929
R 2.	*Solanum nigrum* L.	Kotei-chô, Taihoku	July 10. „
R 3.	*Cosmos bipinnatus* CAV.	College exp. farm.	July 29. „
R 4.	*Trifolium repens* L.	College campus.	Aug. 4. „
R 5.	A certain species of Graminae.	do.	do.
R 6.	Dead leaves of *Dioscorea Batatas* DECNE.	Hotarubashi, Taihoku.	Aug. 11. „
R 7.	*Bidens pilosa* L.	do.	Aug. 16. „
R 8.	*Lactuca chinensis* MAKINO.	Higashisono-chô, Taihoku.	Aug. 15. „
R 9.	*Helianthus tuberosus* L.	Tomita-chô, Taihoku.	Aug. 21. „
R 10.	*Colocasia antiquorum* SCHOTT. var. *esculenta* SCHOTT.	College exp. farm.	Sept. 11. „
R 11.	*Corchorus capsularis* L.	Tomita-chô, Taihoku.	Sept. 20. „
R 12.	*Carica Papaya* L.	College exp. farm.	Sept. 19. „
R 13.	*Houttuynia cordata* THUNB.	Tomita-chô, Taihoku.	Sept. 20. „
R 14.	*Sesbania Sesban* (L) MERRILL.	College exp. farm.	do.
R 15.	*Mosla formosana* MAXIM.	Ryûan, Taihoku.	Sept. 22. „
R 16.	*Saccharum officinarum* L.	College exp. farm.	Oct. 11. „
R 24.	*Trifolium repens* L. †	College campus.	June 3. 1930

† On the affected area sclerotia were very few, but on the culture media typical sclerotia, common to *Sclerotium Rolfsii*, were abundantly formed.

* The sclerotia were sterilized in 1/1000 corrosive sublimate for one minute, and then washed thoroughly with sterilized water.

EXPERIMENTAL PROCEDURE AND OBSERVATION

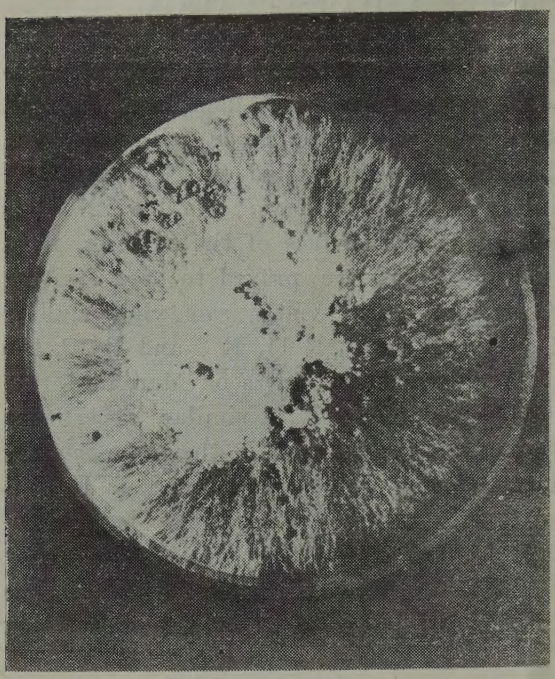
The above-named culture media (slant) were inoculated with a sclerotium of the strains to be studied. All the cultures were kept in a dim and well ventilated cabinet. Temperature was about the same with the room temperatures of our laboratory. The culture was started on Nov. 28, 1929, and the observation was made at intervals of a few days. On Dec. 27 some pure white, powdery masses were noticed on the surface of the cultures, R 14 and R 16. By microscopical examination they were proved to be a fruiting stage of the genus Hypochnus, or Corticium, subsequently somewhat similar fruiting structures were also found on the cultures, R 11, R 3 and R 12, the first one being noticed on Jan. 1, 1930, and the remainders on March 14.

In order to confirm this result more definitely, a similar experiment was performed on Jan. 4, and all the cultures were kept in the same manner as described above. On Feb. 27 some fruiting bodies were found on R 1, R 10, R 14, R 16, and a little later on R 11. There were also some hymenium-like structures noticed on R 4 and R 15, but up to the present time they have never developed to perfection. So far as the present experiment is concerned, the cultures R 11, R 14 and R 16 appear to be readily sporulating strains.

MORPHOLOGY OF THE FUNGI

The young mycelial mass of the fungi is generally snow white with silky-lustre, but becomes more or less dull in old. On the stem of infected plants as well as over the surface of the ground near the plants there are observed numerous characteristic mycelial strands growing outward and branching in an acute manner, thus assuming a fan-shaped appearance or a radiating outgrowth. The same is noticed on cultures as well. Microscopical examination shows that these mycelial strands consist of two types of mycelia. The one is characterized by growing straightly and having broad and elongated cells, 5–7 × 60–100μ, with a clamp connection across a septum, or such connections arranged in pairs on opposite sides, from these connections not infrequently arising new hyphal branches running in parallel or in an acute manner to each other. The other type of hyphae is much narrower, 1.5–2.5μ in width, and grows rather irregularly.

Sclerotia are at first white, simulating white knots with or without stalklets by which they attach to aerial hyphae or substrata. These white interwoven mycelial masses gradually turn yellowish-brown to brown on the outside and finally castaneous brown; generally spherical and smooth, measuring 0.6–1.5 mm. in diameter, but on culture media they are sometimes much irregular and larger in shape. It is observed during the course of sclerotial

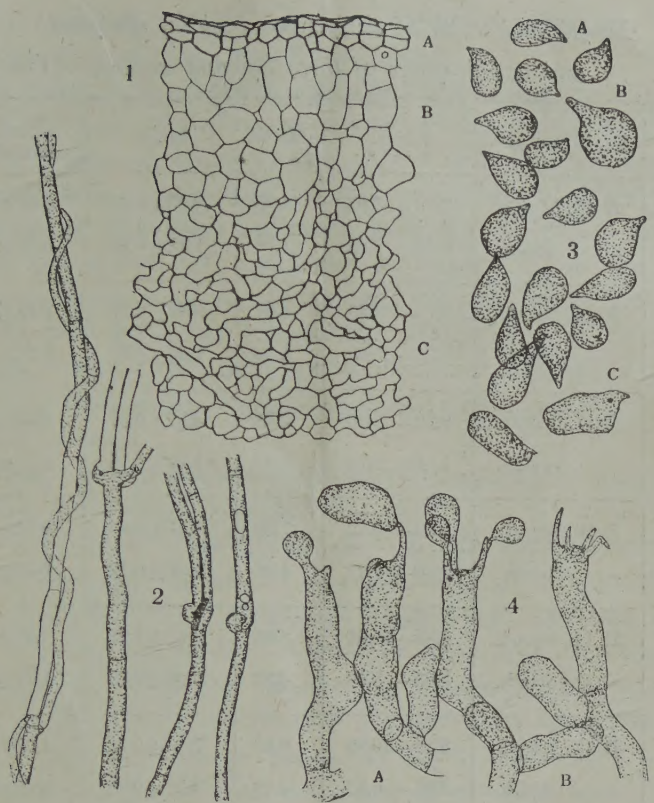


Fertile and sterile forms of R 14 on onion agar. Fruiting structures appearing as white masses on the central part of the surface of the medium as well as on the wall of the vessel.

ripening that a watery drop is frequently exuded from the surface of sclerotia, this part turns darker in color when the drop is dried. Microscopical examination reveals that the peripheral part of the sclerotia consists of pseudoparenchyma, having a rind which is composed of several layers of more or less flattened cells with brown-colored cell wall, and the innermost is filled with prosoplectenchyma.

The fruiting bodies produced on onion agar media are flattened, resupinate, loosely composed, confluenting in pure white mass, the appearance of which is almost identical in essential characteristics of all the strains studied, but somewhat different in details as the strains differ, viz. assuming dendroid form attaching to aerial hyphae, or simulating powdery mildew as often noticed in cultures R 14 and R 1 respectively. A single instance has been observed in the case of naturally affected clovers in which the fruiting bodies developed on the substrata underneath the plants is rather leathery instead of loosely formed. Subhymenial layer is composed of loosely interwoven hyphae (1.5–2.2μ) formed by monopodial branching, each branchlet being septated at its basal part, and becoming gradually broader as it reaches the hymenial layer, and finally each terminal branchlet develops into a basidium, usually septated at its base. Hymenium is also loose and unseparable. Basidia are hyaline, clavate, some of them have two to four sterigmata at the apex which is usually obtuse-oblong in shape, but the majority of them do not bear any sterigmata at all and are shorter in length as compared with the former. Sterigmata are short needle-shaped, sharp-pointed, and taper toward end. Basidiospores are hyaline, smooth-walled, obovate or slightly cylindric, flattened at the inner side and apiculated at the base.

The measurements made of different strains will be shown in the following table.



Explanation of Figure. 1. A part of a section through a sclerotium. A. Rind. B. Pseudoparenchymatous part between the rind and prosoplectenchymatous core. C. Prosoplectenchymatous part. (Leitz ×150). 2. Hyphae taken at the margin of the colony on carrot agar. (Leitz ×150) 3. Various forms of Basidiospores. A. Normal. B and C. Types of abnormal growth (11.68×3.03μ, 11.63×5.11μ resp.). (Leitz ×500) 4. Basidia. A. Two sterigmated. (The larger spore measuring 11.68×5.84μ). B. Normal basidia with four sterigmata. (Leitz ×500)

Length and width of sterigmated basidia, sterigmata and basidiospores of three different strains.

Part of fruiting structures	No. of cultures	Range	Mode	Mean	Standard deviation	No. measured
Basidia	R 16. length.	18.98–41.61	26.28	27.61±3.74	5.54±0.69	50
	width.	4.38 – 7.30	5.84	5.90±0.49	0.73±0.09	50
	R 14. length.	18.98–56.94	28.47	32.85±5.49	8.14±1.15	50
	width.	4.38– 7.30	5.11	5.61±0.51	0.75±0.11	50
	R 11. length.	15.33–30.66	20.81	21.8[illegible]±0.38	1.30±0.13	50
	width.	4.38– 7.30	5.84	5.88±0.43	0.64±0.06	50

Length and width of sterigmated basidia, sterigmata and basidiospores of three different strains.—(Cont.)

Part of fruiting structures	No. of cultures	Range	Mode	Mean	Standard deviation	No. measured
Sterigmata	R 16. length.	2.19 8.03	4.38	4.85±0.63	1.09±0.08	100
	width.	0.83 - 1.83	1.36	1.45±0.14	0.21±0.02	100
	R 14. length.	2.19— 9.49	5.04	5.56±0.77	1.14±0.08	100
	width.	0.71— 2.19	1.43	1.44±0.01	0.17±0.01	100
	R 11. length.	2.19— 7.30	4.38	4.52±0.57	0.85±0.06	100
	width.	1.50— 2.13	1.46	1.54±0.15	0.2[illegible]±0.02	100
Basidiospores	R 16. length.	5.48—10.22	7.30	7.17±0.63	0.94±0.07	100
	width.	2.90— 6.57	4.38	4.80±0.12	0.18±0.01	100
	R 14. length.	5.84—12.41	8.03	8.42±0.95	1.41±0.10	100
	width.	2.65— 8.03	4.38	5.02±0.75	1.11±0.08	100
	R 11. length.	5.11—10.95	6.57	7.15±0.72	1.07±0.08	100
	width.	3.65— 6.57	4.78	4.55±0.42	0.62±0.04	100

IDENTIFICATION OF THE FUNGI

Considering the characteristics mentioned above it is inferred that all the strains examined by the writer may properly be considered as a single species, regardless of some differences observed in some cultures, and it is also learned that the writer's fungi are quite identical with *Sclerotium Rolfsii* in most important particulars, when a comparison is made with the very species described by such authors as SACCARDO (24, 19[illegible]3 and 25), PELTIER (22), TAUBENHAUS (32), TISDALE (35), HIGGINS (13), TAKAHASHI (31), YOKOGI (37) and THOMPSON (34), as well as with the culture designated *Sclerotium Rolfsii* which was isolated by L. S. AJREKER.*

The next attention is naturally turned to the question on the identity of the perfect stage of his fungi studied. In comparison of the writer's with *Hypochnus centrifugus* described by SCHROETER (29), SAWADA (26, 27 and 28) and NAKATA (19 and 20) it was found that the former slightly differs from any of the above authors' by having longer basidia and somewhat larger

* The culture was received through the courtesy of the Lister Institute, London.

spores, but these differences may not be sufficient to warrant a separation of the former from remainders as a distinct species.

Then the question is centered upon the point whether his fungi can be referred to the binomial *Hypochnus centrifugus* as stated by SAWADA, or included in the genus Corticium according to the scheme proposed by such authors as will be mentioned later. *Hypochnus centrifugus* was first described by TULASNE as a perfect stage of *Rhizoctonia centrifuga* LÉVEILLÉ in 186'. In those days, however, there was a lack of unanimity in opinion as to the relationship between the genera Hypochnus and Corticium. In SACCARDO'S (24, 1888) there appears the following difference between the two genera.

Corticium. Fungi resupinat-reflexi, hymenio subceraceo, mycelio imposito, absque strato intermedio, humido saepius molli, sicco rimoso-partito, non pulverulento nec velutino.

Hypochnus. Fungi floccoso-collabentes vel mucedinei resupinati; basidia tetraspora hyphis laxis longis suffulta.

As shown by these descriptions the generic characteristics are mostly expressed on macroscopic natures. Towards the end of the 19th. century, however, such mycologists as KARSTEN*, SCHROETER (29) and MASSEE (17) recognized the significance of the characters of spores for classification of these groups, and introduced this idea to the scheme in classification. Afterwards this was followed and modified by HERTER (11) and other mycologists. At present the general tendency shown by BURT (4, 5 and 6), KILLERMANN (14) and LINDAU-ULBRICH (16) is; the genus Hypochnus being characterized by having rough-walled to echinulated spores which are usually distinctly colored, while the genus Corticium differs from the former in its hyaline and smooth-walled spore and absence of cystidia (according to BURT gloeocystidia are not included herein.). If this scheme is adopted in classification of the writer's fungi, it is logically concluded that the species in question is to be transferred to the genus Corticium and given binomial designated this generic name. Up to the present time there have been noticed two closely related species of Corticium which appear to resemble the writer's fungi, i. e. *Corticium arachnoideum* BERK. and *Corticium centrifugum* (LÉV.) BRES. According to SCHROETER (29), SACCARDO (24, 1901), HERTER (12), OUDEMANS (21) and LINDAU-ULBRICH (16), *Corticium arachnoideum* is considered as a synonym of *Hypochnus centrifugus*. However the writer hesitates to consider them synonymous, since BURT (6) in his extensive studies on a large number of exsiccati, including BERKELEY'S type species of *Corticium arachnoideum*, denied the identity of these two species. On the other hand, as to the relationship between *Hypochnus centrifugus* with *Corticium centrifugum*, such authors as HERTER (12), LIND (15), SAWADA (27 and 28), BURT (6) and LINDAU-ULBRICH (16), considered them identical, whereas SACCARDO (24, 1888 and 1905) and NAKATA

* Cited from Burt (4).

(19) treated them separately, according to the latter author *Hypochnus centrifugus* should not be identified with *Corticium centrifugum*, as the imperfect stage of the latter was referred to the genus Rhizoctonia. As already mentioned, *Hypochnus centrifugus* was first described as the perfect stage of *Rhizoctonia centrifuga*, and further the latter fungus was proposed by BRESADOLA as a synonym of his species *Corticium centrifugum*. If these arguments are admitted, then it naturally follows that *Hypochnus centrifugus* and *Corticium centrifugum* are recognized as being identical with each other. Nevertheless, the matter can not be so simply settled, since the identity of *Rhizoctonia centrifuga* with *Hypochnus centrifugus* is doubtful according to SACCARDO (24, 1901), and moreover in the BRESADOLA's original description no mention was given about the sclerotia except for a brief notice "Sclerotia non vidi". Therefore it is rather difficult at present to refer the perfect stage of the writer's fungi to the binomial *Corticium centrifugum*. It is confirmed, however, that the writer's fungi are quite identical in every details with *Corticium centrifuga* (LÉV.) BRES. described by HERTER (12) and LINDAU-ULBRICH (16), though it is curious that these authors as well as BURT (6) appear to have paid no much attention to the original description of BRESADOLA (3) as to the matter of sclerotia for classification of their fungi. For the time being, therefore, it is rather safe to say that the writer's fungi are identical with HERTER's fungus designated *Corticium centrifugum*, and that in all probability they might be also similar to the type species of BRESADOLA.

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Errata.

P. 165, line 6. For "is" read "are".
„ line 11. For "plant" read "plants".
P. 168, line 27. For "is" read "are".
„ line 34. For "do" read "does".
„ line 35. For "are" read "is".
P. 169, figure, legend. For "×150" and "×500" read "×300" and "×1000", respectively.
„ figure, legend. Line 5. For "11.63" read "11.68".
P. 170, table. Steri mata R 11. For "length" read "length".
P. 171, line 7. For "186" read "1861".
P. 172, line 15. For "centrifuga" read "centrifugum".
P. 173, line last but one. For "it's" read "its".

培養基上に形成せられたる Sclerotium Rolfsii 菌の完全時代に就て（豫報）

後藤和夫

（摘　要）

著者は昨夏以來臺北市に於て菌核病を惹起し居たる *Sclerotium Rolfsii* 菌を十七の異種植物上より採集分離し之を玉葱煎汁寒天に培養研究中、十二月二十七日以來下記五種の菌 R14, R16, R11, R3, R12, に於て子實體を形成せるを認めたり。本菌の子實體に關しては既に澤田氏が自然狀態に於て之を詳細に觀察せるも、人工培養基上にありては今日まで各國にて廣く研究せられたるにも拘らず中田氏によりて突然變異菌に於て認められたる外、未だ何等の確證を得ず。斯く稀有の現象なりしが故にその後繰返へし子實體形成を同一培養基に試みしに R1, R10, R14, R16. R11 等に於て前と同樣なる子實體の形成を認め得たり。就中 R11, R14, R16 の三菌は容易に子實體を形成するもの丶如し。

本菌の形態　菌絲は束狀に結合し純白絹絲光澤を有し被害植物上又は附近地表を走る。之を鏡檢すれば菌絲は無色透明にして二型に分ち得。一は多數の clamp connection を有し特有の分岐法を採る太き氣中菌絲（幅 5－7μ）にして他は細く（幅 1.5－2.5μ）不規則の走向、分岐をなす。菌核は小粒表面平滑栗褐色にして多數被害部附近に生ず。玉葱寒天上に於ても亦同樣なり。菌核の內部は無色透明、その表面近くのものは Pseudoparenchyma よりなりその最外二三層の細胞は稍扁平にしてその膜壁褐色を呈し皮層部を形成す。中心部は Prosoplectenchyma よりなる。培養基上に發達せる結實體は純白色稍扁平粗鬆表面粉狀を呈し、其の系統により或ひは氣中菌絲に懸りて樹枝狀をなし又は基面に粉狀に生ずる等の多少の變異を示す。子實層下の菌絲は幅 1.5－2.2μ 單軸的分岐をなし末端枝は單胞にして擔子囊となる。擔子囊は上面生にして棍棒狀をなし少數のもの丶み其の頂端に 2-4 箇の小柄を有し、この大きさ約 15－57×4.4－7.3μ なり。小柄は約 2－8μ 擔子胞子は無色透明倒卵形平滑、下端に小突起あり。大きさ約 5－12.5×3－8μ。

分類的考察　上記の如き諸形態よりして本菌は其の不完全時代は多くの研究者に依りて認められたる *Sclerotium Rolfsii* に該當す可く、又其の完全時代は Schroeter, 澤田、中田等の記述に依る *Hypochnus centrifugus* 菌に一致すべきものと認む。然れども著者は最近に於ける Thelephoraceae の分類學的傾向より考慮して本菌は Hypochnus に隸屬せしむ可きものに非ずして Corticium に轉移すべきを至當と認む。却說 *Hypochnus centrifugus* に類似する Corticium につき古來多數の研究者に依りその異名とされしものに *Corticium arachnoideum* Berk.

C. centrifugum (Lév.) Bres. とあり。然れども前者は Burt の詳細なる研究によりて別種と認められ後者に關しては Bresadola が同菌を記載せる原著に於て菌核につき唯「菌核は見當らず」とあるのみなれば俄にそれが異名なるや否やを決定するに躊躇せざるを得ず。唯 Herter, Lindau-Ulbrich の記せる *Corticium centrifugum* (Lév.) Bres. には原著に見られざりし菌核に關する記事ありて而も之等はよく本菌と一致せるを認む。更に又一方 Burt は *Corticium centrifugum* を記載するに當り菌核を見ずして *Hypochnus centrifugum* を異名と認め居れり。是等の諸點より著者は本報文に於て本菌は Herter の *Corticium centrifugum* に一致すべきものにして、又恐らくは Bresadola の *Corticium centrifugum* に該當すべきものなるべしと結論せり。